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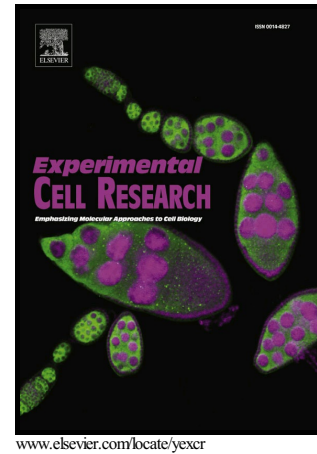
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Endothelial cell-cell adhesion and signaling

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## **Endothelial cell-cell adhesion and signaling**

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**Abstract**

Endothelial cells line blood vessels and provide a dynamic interface between the blood and tissues. They remodel to allow leukocytes, fluid and small molecules to enter tissues during inflammation and infections. Here we compare the signaling networks that contribute to endothelial permeability and leukocyte transendothelial migration, focusing particularly on signals mediated by small GTPases that regulate cell adhesion and the actin cytoskeleton. Rho and Rap GTPase signaling is important for both processes, but they differ in that signals are activated locally under leukocytes, whereas endothelial permeability is a wider event that affects the whole cell. Some molecules play a unique role in one of the two processes, and could therefore be targeted to selectively alter either endothelial permeability or leukocyte transendothelial migration.

## Introduction

Endothelial cells (ECs) form a semi-permeable barrier to separate the blood stream from the underlying organs and tissues and control the transport of fluids, solutes and cells across blood vessel walls. The barrier is mediated by endothelial cell-cell adhesions including tight junctions (TJ), adherens junctions (AJ) and a variety of other adhesion molecules including PECAM-1 and nectins, which are connected to the actin cytoskeleton via different adaptor molecules (Fig. 1). The architecture and the composition of cell-cell junctions varies between vascular beds depending on the organ-specific requirements [1, 2]. In addition to maintaining adhesion between ECs, cell-cell junctions control vascular permeability and leukocyte migration via a complex balance between multiple signaling molecules (Fig. 1, Fig. 2) [3, 4].

TJs regulate the diffusion of ions and polar solutes and block penetration of large macromolecules across ECs. TJs are formed by the homophilic cell-cell adhesion molecules occludin, claudins and junctional adhesion molecules (JAMs) (Fig. 1) [5, 6]. Claudins are the principal barrier-forming proteins, in particular claudin-5 is critical for endothelial permeability *in vivo* and *in vitro* [7]. Occludin and claudins are linked to zonula occludens (ZO)-1, ZO-2, ZO-3, cingulin and other protein complexes, which mediate the interaction between the adhesion molecules and actin filaments [5]. The JAM family is composed of three closely related proteins JAM-A, -B and -C, and by the coxsackie and adenovirus receptor (CAR). The JAMs mediate endothelial cell-cell interaction and regulate leukocyte transendothelial migration (TEM) [8]. Both JAMs and CAR regulate permeability by supporting TJ function and assembly [9].

VE-cadherin is the key transmembrane component of endothelial AJs and is expressed only in ECs [10]. VE-cadherin together with PECAM-1 initiates and maintains endothelial cell-cell contact, holding the ECs together to give mechanical support to the endothelium and provide endothelial junction stability [11, 12]. AJs can regulate expression of TJ components and TJ organization follows AJ formation [11, 13]. In addition, AJs and TJs are interconnected [14], for example ZO proteins crosstalk with AJs [15]. VE-cadherin is linked indirectly via its cytoplasmic tail to actin filaments by a complex of proteins including  $\alpha$ - and  $\beta$ -catenins, plakoglobin ( $\gamma$ -catenin), p120-catenin, vinculin and  $\alpha$ -actinin (Fig. 1) [16], which are vital for junctional stability and also for the dynamic opening and closing of junctions [17].

Here we discuss the roles of endothelial cell-cell junctions in signaling leading to changes to endothelial permeability and during leukocyte transendothelial migration (TEM), with a particular focus on small GTPases.

### **Regulation of small GTPases**

Several members of the Ras superfamily of small GTPases contribute to endothelial cell-cell adhesion and hence regulate endothelial permeability and/or leukocyte TEM. These include Rap1, Rap2 and several Rho family GTPases (Fig. 2, Fig. 3). Most small GTPases cycle between an inactive GDP-bound conformation and an active GTP-bound conformation. This cycling is regulated by guanine nucleotide exchange factors (GEFs), which catalyse the exchange of GDP for GTP, thereby activating the proteins, and by GTPase-activating proteins (GAPs), which stimulate GTP hydrolysis and inactivate the proteins. The interaction between small GTPases and their downstream effectors often requires their localisation to membranes, which is mediated by post-translational modification by lipids (prenylation and/or palmitoylation) [18, 19]. For example, geranylgeranylation of Rho GTPases is required for thrombin-induced permeability in ECs [20]. Some small GTPases are inhibited by binding to proteins that extract them from membranes, including guanine nucleotide dissociation inhibitors (GDIs) and 14-3-3 proteins [21, 22]. Small GTPases are rapidly activated by cell-surface receptors, and a single GTPase can interact with multiple downstream targets to induce a range of cellular responses in a spatio-temporal fashion, depending on the stimulus and cell type [19].

### **Signaling to endothelial junctions in vascular permeability**

Endothelial cell-cell junctions remodel dynamically in response to multiple extracellular stimuli, which transiently increase or decrease endothelial permeability and thereby regulate the entry of polar solutes and macromolecules into the tissues from the blood stream (Fig. 2) [4]. For example, pro-inflammatory stimuli such as thrombin, histamine and TNF $\alpha$  increase permeability, whereas anti-inflammatory mediators including sphingosine 1-phosphate (S1P) and angiopoietin-1 decrease permeability [23]. Increased vascular barrier leakage is associated with disruption of endothelial junctions, in particular AJs [10], and occurs in diseases such as cancer, atherosclerosis, diabetes, hypertension, inflammation, and ischemia [24, 25]. In addition, cerebral cavernous malformations (CCMs) are inherited

or sporadic diseases that are associated with disorganization of venous brain microvessel junctions and increased permeability [26].

#### *Signals that increase endothelial permeability*

Pro-inflammatory stimuli increase permeability through a combination of changes to AJ, TJ and the associated actin cytoskeleton (Fig. 2). AJ composition and integrity is regulated by changes in phosphorylation of junctional proteins. For example, thrombin activates the serine/threonine kinase protein kinase C (PKC) $\alpha$ , which induces VE-cadherin complex disassembly by phosphorylating p120ctn [27]. In VEGF-stimulated ECs, Src mediates Rac1 activation, leading to VE-cadherin phosphorylation via the serine/threonine kinase PAK, and thereby increasing endothelial permeability [28]. LPS activates the Rab family GTPase Rab5, which mediates VE-cadherin internalization resulting in increased permeability [29].

Tyrosine phosphorylation of AJ components is also associated with increased permeability. In unstimulated ECs, receptor and cytoplasmic protein tyrosine phosphatases (PTPs) localize to endothelial AJs and de-phosphorylate AJ components to reduce permeability [17, 30, 31]. For example, the tyrosine phosphatase SHP2 associates with  $\beta$ -catenin in VE-cadherin/p120/plakoglobin complexes [32]. Depletion of SHP2 enhances phosphorylation of AJ complex components leading to increased permeability in response to thrombin [32, 33]. TNF $\alpha$  increases tyrosine phosphorylation of VE-cadherin to enhance vascular permeability [34, 35]. VE-cadherin Tyr685 phosphorylation is required for VEGF- and histamine-induced permeability in vivo [36]. Furthermore, phosphorylation of VE-cadherin on Tyr658 and Tyr685 was reported to be required for increased permeability and endocytosis of VE-cadherin in response to bradykinin and histamine [37].

Endothelial cell-cell contacts are linked to the actin cytoskeleton, which is central to junction stability and contributes to changes in vascular permeability [38]. Permeability-inducing factors destabilize cell-cell junctions by changing actin bundle orientation, which switch from being parallel to junctions to being perpendicular. Perpendicular actin filaments, including stress fibers, impose mechanical force on junctions, and disrupt their integrity [39-41]. RhoA induces stress fibers and increases contractility leading to higher vascular permeability in response to a variety of extracellular stimuli including thrombin and histamine (Fig. 2). RhoA activates the serine/threonine kinase ROCK, which phosphorylates the MYPT1 subunit of the trimeric myosin light chain (MLC) phosphatase, thereby inhibiting

its enzymatic activity [42, 43]. Subsequent increased MLC phosphorylation leads to increased actomyosin contractility. RhoA is required for increased permeability in response to several stimuli including thrombin and TNF $\alpha$  [44]. Interestingly, RhoB and RhoC are also activated by thrombin [45], and RhoB promotes sustained thrombin-induced contraction [46]. By contrast, RhoA activation by angiopoietin-1 leads to cell-cell junction stabilization and inhibits VEGF-induced permeability, and this response is dependent on the Rho-activated formin mDia1 but not ROCK [47]. This suggests that the effect of RhoA on junctions depends on whether it activates ROCK, promoting actomyosin contractility, or on mDia1, which stimulates actin polymerization [48].

Increased permeability often reflects loss of TJ proteins from junctions, which is linked to enhanced RhoA/ROCK signaling. For example, ROCK induces phosphorylation of claudin-5 and occludin [49]. TNF $\alpha$  increases permeability by inducing a decrease in claudin-5 at junctions via ROCK1 and ROCK2 [50]. LPS induces loss of TJ proteins and increased endothelial permeability via activation of RhoA by p115RhoGEF [51]. In the TJ complex, ZO-1 is bound to cingulin which is linked to the actin cytoskeleton (Fig. 1). Cingulin promotes endothelial barrier function *in vitro* and *in vivo*, in part by interacting with and inhibiting the RhoA GEF-H1 [52, 53], whereas the cingulin-like protein JACOP associates with p114RhoGEF to maintain endothelial TJs and AJs, presumably by stimulating RhoA activity at cell-cell junctions [15]. This indicates that the site of RhoA activation, and which of its targets it stimulates, determines whether it inhibits or stimulates junction disruption.

#### *Signals that reduce permeability*

Stimuli that reduce permeability and promote endothelial barrier function generally act through three small GTPases, Rap1, Rac1 and Cdc42, and as described above in some cases RhoA, which act together through inter-connected signaling networks (Fig. 2). For example, thrombin initially increases endothelial permeability through RhoA, but also increases S1P generation, which then stimulates Rac1 activation resulting in enhanced barrier integrity to reverse the thrombin-induced permeability [54].

Endothelial junctional integrity is enhanced by stimuli that elevate cAMP levels, such as adrenomedullin, prostacyclin, prostaglandin E<sub>2</sub>, and  $\beta$ -adrenergic agonists, which reduce EC permeability [55]. cAMP directly activates the RapGEF Epac, which activates Rap1 [56].

There are two Rap1 proteins, Rap1a and Rap1b. Rap1b is most highly expressed in ECs, but



Rap1a depletion reduces EC barrier function more than Rap1b depletion, which could be explained by its colocalization with VE-cadherin at AJs [57].

Rap1 acts through multiple pathways to promote VE-cadherin-mediated adhesion and maintain barrier function [56]. First, it leads to activation of Rac1 and Cdc42, which in turn strengthen endothelial cell-cell junctions. For example, Epac1/Rap1 act via the Rac GEFs Tiam1 and Vav2 to promote Rac1 activation [55]. In addition, circulating erythrocytes or platelets release S1P that activates Rac1 downstream of Rap1 via Akt, leading to endothelial barrier stabilization [58, 59]. Rap1 also promotes the assembly of a junctional mechanosensing complex of PECAM1, VE-cadherin and VEGFR2 in response to shear stress, which then activates Rac1 via Vav2 and Tiam1 to increase barrier function [60-62]. Finally, Rap1 activates Cdc42 via the RhoGEF FGD5 to promote cell-cell junction stabilization [63, 64].

In addition to activating Rac1 and Cdc42, Rap1 acts via several mechanisms to reduce RhoA/ROCK activity resulting in increased endothelial barrier function (Fig. 2). It acts via its effector Rasip1, which recruits the RhoGAP ARHGAP29 to inhibit RhoA and ROCK activity [65, 66]. Rasip1 also decreases stress fiber formation and endothelial permeability by direct interaction with the transmembrane receptor heart of glass (HEG1) [67]. Furthermore, Rap1 controls the endothelial barrier by recruiting its effector CCM1/KRIT1 to EC junctions, which reduces stress fibers and RhoA activity in ECs [68, 69]. In contrast to Rap1, Rap2 depletion increases endothelial barrier resistance, although the mechanism whereby Rap2 alters barrier function is not known [70].

Rac1 increases EC junction stability and hence reduces permeability, both by stimulating extension of lamellipodia to close intercellular gaps and by inducing assembly of cortical F-actin bundles and reducing actomyosin tension [71, 72]. In addition, shear stress acts via VE-cadherin, Tiam1 and Rac1 to reduce the level of tyrosine phosphorylation on occludin, leading to barrier enhancement [73]. Although the tyrosine phosphatase involved in this pathway has not been identified, Angiopoietin-1 reduces occludin tyrosine phosphorylation via the protein tyrosine phosphatase N-2 and promotes occludin interaction with ZO-1 to enhance TJs [74], and thus it will be interesting to test if this phosphatase acts downstream of Rac1.

Rac1 acts through several downstream effectors to mediate junction stabilization, including IQGAP1, which binds to Rac1 and Cdc42, and interacts with activators of actin

polymerization (N-WASP, Arp2/3 complex) to promote AJ assembly [75, 76]. By contrast, Rac1 has been reported to increase permeability downstream of VEGF via PAK-mediated phosphorylation of a highly conserved motif within the intracellular tail of VE-cadherin, Ser665, resulting in VE-cadherin internalization [47]. VEGF activates VEGFR2 which associates with VE-cadherin and activates Rac1 via Src and the RacGEF Vav2 [47]. VEGF can also act via the phosphatidylinositol (3,4,5)-trisphosphate-dependent Rac exchanger 1 (P-Rex1) to activate Rac1 and increase permeability [77]. It is therefore likely that the effect of Rac1 on permeability depends on the cellular context.

As well as being activated downstream of Rap1, Rac1 is locally activated at cell-cell junctions, in part via the RhoGEFs Trio and Tiam1, which interact with VE-cadherin [44, 78-80]. On the other hand, inflammatory mediators such as LPS, TNF $\alpha$ , angiotensin 2 and thrombin reduce Rac1 activity resulting in junction opening and increased permeability [81]. RhoB inhibits Rac1 activity at junctions by reducing trafficking of Rac1 to cell-cell junctions. RhoB expression is induced by the inflammatory cytokines TNF $\alpha$  and IL-1 $\beta$ , and promotes sustained EC contraction upon thrombin exposure in the context of inflammation [46]. Like Rac1, Cdc42 is important for maintaining AJs [82, 83] [84]. For example, Cdc42 restores endothelial barrier function upon thrombin stimulation [85]. Increased endothelial permeability induced by loss or destabilization of VE-cadherin increases Cdc42 activity [84, 86], which presumably promotes junctional restoration. In vivo, Cdc42 is required for endothelial cell-cell adhesion during development, and is necessary for endothelial polarity [87].

Cdc42 acts via several mechanisms to stimulate AJ assembly. It promotes the post-Golgi transport of VE-cadherin to AJs [88] and controls  $\alpha$ -catenin binding with  $\beta$ -catenin and interaction of the VE-cadherin complex with the actin cytoskeleton [84]. In addition, Cdc42 controls actin organization through two pathways: first via its effectors PAK2 and PAK4 that regulate junctional actomyosin, and second via N-WASP that is required for junctional actin assembly [87]. Cdc42 has also been reported to act through the atypical protein kinase C PKC $\iota$  to mediate endothelial cell-cell adhesion in vivo [89].

Overall, endothelial permeability reflects the combined actions of multiple signaling molecules, including small GTPases, kinases and phosphatases, which directly modify junctional molecules as well as act on the associated actin cytoskeleton to alter junctional integrity (Fig. 2).

### Signaling in endothelial junctions and leukocyte transendothelial migration

Leukocyte TEM is essential both in immunosurveillance and during tissue damage or infection [90, 91]. TEM is a unique process in which a leukocyte squeezes itself through the endothelium, and involves dynamic signaling between leukocytes and ECs leading to the changes in morphology required for TEM (Fig. 30).

Leukocytes cross the endothelium via a multistep cascade involving leukocyte capture, rolling, adhesion, and crawling on ECs followed by leukocyte migration across the endothelium in the presence of shear forces exerted by blood flow (Fig. 3) [92-94].

Leukocytes can cross the endothelium through two different routes: paracellular, through the cell-cell junctions, or transcellular, through the endothelial cell body [95, 96]. The choice of route depends on the transmigrating cell type, the site of TEM and the type of vascular endothelium [97, 98]. Furthermore, the route of diapedesis depends on endothelial junctional integrity: T-cells are more likely to take a transcellular route when junctions are strengthened [99].

Several adhesion molecules expressed by ECs contribute to TEM, including ICAM-1 and/or VCAM-1 on the luminal surface and PECAM-1 and/or JAMs on the basolateral membranes [8, 93, 100]. The expression and regulation of these molecules depends on the shear stress of the blood flow, proinflammatory cytokines and chemokines, the vascular bed and the stiffness of the tissue [101, 102].

#### *Leukocyte crawling and endothelial extension of docking structures*

Leukocytes initially crawl on ECs before arresting and transmigrating. The leukocyte integrin LFA-1/ $\alpha$ L $\beta$ 2 interaction with ICAM-1 activates RhoA [94]. RhoA promotes stiffening of the endothelial surface, enhancing leukocyte migration over it and hence the ability of leukocytes to find a site for TEM. ICAM-1 activates RhoA through the RhoGEF LARG (Leukemia-associated Rho GEF, ARHGEF12), and depletion of endothelial LARG reduces leukocyte crawling on ECs [103].

ECs often extend dynamic microvillus-like protrusions known as docking structures or trans migratory cups around adherent leukocytes (Fig. 3) [94]. These structures contribute to TEM, probably by increasing leukocyte-endothelium contact area with minimal barrier

disruption and to help leukocytes extend protrusions to cross the endothelium [104]. Initially ICAM-1 and VCAM-1 cluster in ring-like structures around the leukocyte, followed by apical membrane protrusion. Anchorage of ICAM-1 to the actin cytoskeleton via adaptor proteins, such as filamin and cortactin, is essential for formation of ICAM-1 rings and subsequent leukocyte TEM [105-107].

Rac1 is required for ICAM-1 clustering, while RhoG controls membrane protrusion (Fig. 3). RhoG is activated by ICAM-1 binding to the RhoG-specific GEF SGEF [108]. ICAM-1 also associates with and activates the GEF Trio, which activates Rac1 and then RhoG via its two different RhoGEF domains [109]. Interestingly, Trio expression is strongly upregulated by TNF $\alpha$  stimulation, suggesting docking structure assembly is coupled to induction of ICAM-1 and VCAM-1 [109].

#### *Crossing the endothelium and closing the transmigration pore*

For paracellular TEM, two signaling events occur once the leukocyte is anchored to the endothelium: local VE-cadherin internalization via clathrin-coated vesicles and influx of membrane and membrane proteins including PECAM-1 from a lateral border recycling compartment (LBRC) to the TEM site [110-113].

Junction opening involves VCAM-1 signaling to VE-cadherin. VCAM-1 interaction with the leukocyte integrin VLA-4 induces Rac1 activation and subsequent production of intracellular reactive oxygen species (ROS) by NADPH oxidase [114]. This in turn activates the proline-rich tyrosine kinase (Pyk2) [115] which then phosphorylates VE-cadherin on Tyr658 and Tyr731 resulting in local loss of VE-cadherin function, junction opening and increased TEM (Fig. 30 [36, 116-118]. Inhibition of this pathway, through the PI3-kinase p110 $\alpha$ , Pyk2 and Rac1, reduces TEM [114, 118-120]. Rac1, ROS and Pyk2 also induce VE-PTP dissociation from VE-cadherin, leading to increased tyrosine phosphorylation of VE-cadherin and VE-cadherin internalization thereby facilitating transmigration [3, 31, 113, 121]. Furthermore, endothelial signaling stimulates junctional actomyosin contractility and acts on cell-cell junctional proteins, leading to localised and transient junctional disassembly essential for leukocyte TEM [94, 122].

Multiple proteins are associated with the LBRC fraction during TEM including PECAM-1, which is important for TEM [123, 124]. PECAM-1 homotypic interaction starts the recruitment of the LBRC to the site of leukocyte interaction. This is linked to localised

recruitment of the  $\text{Ca}^{2+}$  channel TRPC6, which increases intracellular  $\text{Ca}^{2+}$  and is required for the final stage of TEM, similar to PECAM-1 [125]. Increased intracellular  $\text{Ca}^{2+}$  triggers actomyosin contractility by myosin light chain kinase (MLCK), which is required for TEM [102]. PECAM-1 is also involved in transcellular TEM, since ICAM-1 clustering on ECs is not sufficient to promote the transcellular pathway if PECAM-1 is blocked [126].

Endothelial RhoA is activated locally by ICAM-1 during TEM through recruitment of the RhoGEFs LARG and Ect2 [45, 97]. RhoA activity is highest during the final stage of extravasation, and mediates endothelial F-actin remodelling to form ring structures around transmigrating leukocytes, which both prevent vascular leakage during leukocyte diapedesis and promote pore closure and transmigration [97].

### Conclusions and future perspectives

Endothelial permeability and leukocyte TEM involve similar intracellular signaling mechanisms, including Rho and Rap GTPase signaling, cell-cell junction and F-actin remodelling, and thus it has been difficult to separate the two processes mechanistically. However, a key difference between them is when and where the signals occur in ECs. For leukocyte TEM, receptors are activated locally in ECs beneath and around the leukocyte [94], whereas for endothelial permeability the receptors are generally activated across the whole EC plasma membrane and/or cell-cell junctions [4]. In addition, TEM does not involve an increase in vascular permeability, rather that mechanisms are in place to ensure minimal leakage [36, 97, 127]. Moreover, stimuli leading to vascular permeability generally act within minutes [23], whereas leukocyte TEM during inflammation requires upregulation of leukocyte-binding receptors on ECs [91].

Although several signaling mechanisms are similar between endothelial permeability and TEM, there are also signals that differ, which could be manipulated to inhibit one or the other process. For example, differences in VE-cadherin tyrosine phosphorylation between the two processes have been reported [36, 128]. Future research should identify how these phosphorylation sites mediate local versus global changes to junctions in ECs. In addition, RhoG is so far only implicated in TEM [108]. On the other hand, Rap1, RhoA and Rac1 are involved in both processes. It is likely, however, that they are part of different protein complexes because the transmembrane receptors involved in permeability and TEM are different. Although several GEFs have been identified to contribute to permeability and

TEM, future research should aim to identify which downstream targets and GAPs of Rho and Rap GTPases are critical to vascular permeability versus leukocyte TEM.

In contrast to leukocyte TEM, comparatively little is known about the contribution of EC signaling to cancer TEM. Multiple receptors on ECs have been implicated in mediating cancer cell TEM, but little is known of their mechanistic roles beyond simply facilitating adhesion [129]. Recently, Ephrin-1 was identified as a ligand on ECs that stimulates tyrosine phosphorylation of EphA2 on cancer cells, inducing cell-cell repulsion and leading to decreased TEM of breast cancer cells [130]. It will be interesting to determine how other receptors contribute to dynamic signaling between cancer cells and ECs.

In conclusion, changes to vascular permeability and leukocyte TEM are orchestrated by a combination of small GTPases, protein kinases and phosphatases, which coordinate changes to endothelial cell-cell junctions with the actin cytoskeleton. Targeting these signaling molecules could be used to reduce inflammation and auto-immune diseases.

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#### **References**

1. Engelhardt, B., and Wolburg, H. (2004). Mini-review: Transendothelial migration of leukocytes: through the front door or around the side of the house? *Eur J Immunol* 34, 2955-2963.
2. Wolburg, H., and Lippoldt, A. (2002). Tight junctions of the blood–brain barrier: development, composition and regulation. *Vascular Pharmacology* 38, 323-337.
3. Goswami, D., and Vestweber, D. (2016). How leukocytes trigger opening and sealing of gaps in the endothelial barrier. *F1000Res* 5.
4. Claesson-Welsh, L. (2015). Vascular permeability--the essentials. *Ups J Med Sci* 120, 135-143.
5. Balda, M.S., and Matter, K. (2016). Tight junctions as regulators of tissue remodelling. *Curr Opin Cell Biol* 42, 94-101.
6. Radeva, M.Y., and Waschke, J. (2017). Mind the gap: mechanisms regulating the endothelial barrier. *Acta Physiol (Oxf)*.

7. Haseloff, R.F., Dithmer, S., Winkler, L., Wolburg, H., and Blasig, I.E. (2015). Transmembrane proteins of the tight junctions at the blood-brain barrier: structural and functional aspects. *Semin Cell Dev Biol* 38, 16-25.
8. Reglero-Real, N., Colom, B., Bodkin, J.V., and Nourshargh, S. (2016). Endothelial Cell Junctional Adhesion Molecules: Role and Regulation of Expression in Inflammation. *Arterioscler Thromb Vasc Biol* 36, 2048-2057.
9. Luissint, A.C., Nusrat, A., and Parkos, C.A. (2014). JAM-related proteins in mucosal homeostasis and inflammation. *Semin Immunopathol* 36, 211-226.
10. Dejana, E., and Vestweber, D. (2013). The role of VE-cadherin in vascular morphogenesis and permeability control. *Prog Mol Biol Transl Sci* 116, 119-144.
11. Dejana, E., Orsenigo, F., Molendini, C., Baluk, P., and McDonald, D.M. (2009). Organization and signaling of endothelial cell-to-cell junctions in various regions of the blood and lymphatic vascular trees. *Cell Tissue Res* 335, 17-25.
12. Lertkietmongkol, P., Liao, D., Mei, H., Hu, Y., and Newman, P.J. (2016). Endothelial functions of platelet/endothelial cell adhesion molecule-1 (CD31). *Curr Opin Hematol* 23, 253-259.
13. Dejana, E., and Giampietro, C. (2012). Vascular endothelial-cadherin and vascular stability. *Curr Opin Hematol* 19, 218-223.
14. Taddei, A., Giampietro, C., Conti, A., Orsenigo, F., Breviario, F., Pirazzoli, V., Potente, M., Daly, C., Dimmeler, S., and Dejana, E. (2008). Endothelial adherens junctions control tight junctions by VE-cadherin-mediated upregulation of claudin-5. *Nat Cell Biol* 10, 923-934.
15. Tornavaca, O., Chia, M., Dufton, N., Almagro, L.O., Conway, D.E., Randi, A.M., Schwartz, M.A., Matter, K., and Balda, M.S. (2015). ZO-1 controls endothelial adherens junctions, cell-cell tension, angiogenesis, and barrier formation. *J Cell Biol* 208, 821-838.
16. Ebnet, K. (2008). Organization of multiprotein complexes at cell-cell junctions. *Histochem Cell Biol* 130, 1-20.
17. Vestweber, D. (2008). VE-cadherin: the major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. *Arterioscler Thromb Vasc Biol* 28, 223-232.
18. Wang, M., and Casey, P.J. (2016). Protein prenylation: unique fats make their mark on biology. *Nat Rev Mol Cell Biol* 17, 110-122.



19. Hodge, R.G., and Ridley, A.J. (2016). Regulating Rho GTPases and their regulators. *Nat Rev Mol Cell Biol* 17, 496-510.
20. Xiao, H., Qin, X., Ping, D., and Zuo, K. (2013). Inhibition of Rho and Rac geranylgeranylation by atorvastatin is critical for preservation of endothelial junction integrity. *PLoS One* 8, e59233.
21. Garcia-Mata, R., Boulter, E., and Burridge, K. (2011). The 'invisible hand': regulation of RHO GTPases by RHOGDIs. *Nat Rev Mol Cell Biol* 12, 493-504.
22. Riou, P., Kjaer, S., Garg, R., Purkiss, A., George, R., Cain, R.J., Bineva, G., Reymond, N., McColl, B., Thompson, A.J., et al. (2013). 14-3-3 proteins interact with a hybrid prenyl-phosphorylation motif to inhibit G proteins. *Cell* 153, 640-653.
23. Mehta, D., and Malik, A.B. (2006). Signaling mechanisms regulating endothelial permeability. *Physiol Rev* 86, 279-367.
24. Dejana, E., Tournier-Lasserre, E., and Weinstein, B.M. (2009). The control of vascular integrity by endothelial cell junctions: molecular basis and pathological implications. *Dev Cell* 16, 209-221.
25. Park-Windhol, C., and D'Amore, P.A. (2016). Disorders of Vascular Permeability. *Annu Rev Pathol* 11, 251-281.
26. Lampugnani, M.G., Malinverno, M., and Dejana, E. (2017). Endothelial cell disease: emerging knowledge from cerebral cavernous malformations. *Curr Opin Hematol*.
27. Vandenbroucke St Amant, E., Tauseef, M., Vogel, S.M., Gao, X.P., Mehta, D., Komarova, Y.A., and Malik, A.B. (2012). PKC $\alpha$  activation of p120-catenin serine 879 phospho-switch disassembles VE-cadherin junctions and disrupts vascular integrity. *Circ Res* 111, 739-749.
28. Gavard, J., Patel, V., and Gutkind, J.S. (2008). Angiopoietin-1 prevents VEGF-induced endothelial permeability by sequestering Src through mDia. *Dev Cell* 14, 25-36.
29. Yang, J., Yao, W., Qian, G., Wei, Z., Wu, G., and Wang, G. (2015). Rab5-mediated VE-cadherin internalization regulates the barrier function of the lung microvascular endothelium. *Cell Mol Life Sci* 72, 4849-4866.
30. Wallez, Y., Cand, F., Cruzalegui, F., Wernstedt, C., Souchelnytskyi, S., Vilgrain, I., and Huber, P. (2007). Src kinase phosphorylates vascular endothelial-cadherin in response to vascular endothelial growth factor: identification of tyrosine 685 as the unique target site. *Oncogene* 26, 1067-1077.



31. Koppers, V., Vockel, M., Nottebaum, A.F., and Vestweber, D. (2014). Phosphatases and kinases as regulators of the endothelial barrier function. *Cell Tissue Res* 355, 577-586.
32. Timmerman, I., Hoogenboezem, M., Bennett, A.M., Geerts, D., Hordijk, P.L., and van Buul, J.D. (2012). The tyrosine phosphatase SHP2 regulates recovery of endothelial adherens junctions through control of beta-catenin phosphorylation. *Mol Biol Cell* 23, 4212-4225.
33. Ukropec, J.A., Hollinger, M.K., Salva, S.M., and Woolkalis, M.J. (2000). SHP2 association with VE-cadherin complexes in human endothelial cells is regulated by thrombin. *J Biol Chem* 275, 5983-5986.
34. Angelini, D.J., Hyun, S.W., Grigoryev, D.N., Garg, P., Gong, P., Singh, I.S., Passaniti, A., Hasday, J.D., and Goldblum, S.E. (2006). TNF-alpha increases tyrosine phosphorylation of vascular endothelial cadherin and opens the paracellular pathway through fyn activation in human lung endothelia. *Am J Physiol Lung Cell Mol Physiol* 291, L1232-1245.
35. Nwariaku, F.E., Liu, Z., Zhu, X., Turnage, R.H., Sarosi, G.A., and Terada, L.S. (2002). Tyrosine phosphorylation of vascular endothelial cadherin and the regulation of microvascular permeability. *Surgery* 132, 180-185.
36. Wessel, F., Winderlich, M., Holm, M., Frye, M., Rivera-Galdos, R., Vockel, M., Linnepe, R., Ipe, U., Stadtmann, A., Zarbock, A., et al. (2014). Leukocyte extravasation and vascular permeability are each controlled in vivo by different tyrosine residues of VE-cadherin. *Nat Immunol* 15, 223-230.
37. Orsenigo, F., Giampietro, C., Ferrari, A., Corada, M., Galaup, A., Sigismund, S., Ristagno, G., Maddaluno, L., Koh, G.Y., Franco, D., et al. (2012). Phosphorylation of VE-cadherin is modulated by haemodynamic forces and contributes to the regulation of vascular permeability in vivo. *Nat Commun* 3, 1208.
38. van Buul, J.D., and Timmerman, I. (2016). Small Rho GTPase-mediated actin dynamics at endothelial adherens junctions. *Small GTPases* 7, 21-31.
39. Noda, K., Zhang, J., Fukuhara, S., Kunitomo, S., Yoshimura, M., and Mochizuki, N. (2010). Vascular endothelial-cadherin stabilizes at cell-cell junctions by anchoring to circumferential actin bundles through  $\alpha$ - and  $\beta$ -catenins in cyclic AMP-Epac-Rap1 signal-activated endothelial cells. *Mol Biol Cell* 21, 584-596.
40. Millan, J., Cain, R.J., Reglero-Real, N., Bigarella, C., Marcos-Ramiro, B., Fernandez-Martin, L., Correias, I., and Ridley, A.J. (2010). Adherens junctions connect stress fibres between adjacent endothelial cells. *BMC Biol* 8, 11.

41. Hirano, M., and Hirano, K. (2016). Myosin di-phosphorylation and peripheral actin bundle formation as initial events during endothelial barrier disruption. *Sci Rep* 6, 20989.
42. Sun, H., Breslin, J.W., Zhu, J., Yuan, S.Y., and Wu, M.H. (2006). Rho and ROCK signaling in VEGF-induced microvascular endothelial hyperpermeability. *Microcirculation* 13, 237-247.
43. van Nieuw Amerongen, G.P., Beckers, C.M., Achekar, I.D., Zeeman, S., Musters, R.J., and van Hinsbergh, V.W. (2007). Involvement of Rho kinase in endothelial barrier maintenance. *Arterioscler Thromb Vasc Biol* 27, 2332-2339.
44. Beckers, C.M., van Hinsbergh, V.W., and van Nieuw Amerongen, G.P. (2010). Driving Rho GTPase activity in endothelial cells regulates barrier integrity. *Thromb Haemost* 103, 40-55.
45. Reinhard, N.R., van Helden, S.F., Anthony, E.C., Yin, T., Wu, Y.I., Goedhart, J., Gadella, T.W., and Hordijk, P.L. (2016). Spatiotemporal analysis of RhoA/B/C activation in primary human endothelial cells. *Sci Rep* 6, 25502.
46. Marcos-Ramiro, B., Garcia-Weber, D., Barroso, S., Feito, J., Ortega, M.C., Cernuda-Morollon, E., Reglero-Real, N., Fernandez-Martin, L., Duran, M.C., Alonso, M.A., et al. (2016). RhoB controls endothelial barrier recovery by inhibiting Rac1 trafficking to the cell border. *J Cell Biol* 213, 385-402.
47. Gavard, J., and Gutkind, J.S. (2006). VEGF controls endothelial-cell permeability by promoting the  $\beta$ -arrestin-dependent endocytosis of VE-cadherin. *Nat Cell Biol* 8, 1223-1234.
48. Kuhn, S., and Geyer, M. (2014). Formins as effector proteins of Rho GTPases. *Small GTPases* 5, e29513.
49. Yamamoto, M., Ramirez, S.H., Sato, S., Kiyota, T., Cerny, R.L., Kaibuchi, K., Persidsky, Y., and Ikezu, T. (2008). Phosphorylation of claudin-5 and occludin by rho kinase in brain endothelial cells. *Am J Pathol* 172, 521-533.
50. Clark, P.R., Kim, R.K., Poer, J.S., and Kluger, M.S. (2015). Tumor necrosis factor disrupts claudin-5 endothelial tight junction barriers in two distinct NF- $\kappa$ B-dependent phases. *PLoS One* 10, e0120075.
51. Xiaolu, D., Jing, P., Fang, H., Lifan, Y., Liwen, W., Ciliu, Z., and Fei, Y. (2011). Role of p115RhoGEF in lipopolysaccharide-induced mouse brain microvascular endothelial barrier dysfunction. *Brain Res* 1387, 1-7.

52. Tian, Y., Gawlak, G., Tian, X., Shah, A.S., Sarich, N., Citi, S., and Birukova, A.A. (2016). Role of Cingulin in Agonist-induced Vascular Endothelial Permeability. *J Biol Chem* 291, 23681-23692.
53. Schossleitner, K., Rauscher, S., Groger, M., Friedl, H.P., Finsterwalder, R., Haberktheuer, A., Sibilia, M., Brostjan, C., Fodinger, D., Citi, S., et al. (2016). Evidence that cingulin regulates endothelial barrier function in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 36, 647-654.
54. Mahajan-Thakur, S., Bohm, A., Jedlitschky, G., Schror, K., and Rauch, B.H. (2015). Sphingosine-1-Phosphate and Its receptors: a mutual link between blood coagulation and inflammation. *Mediators Inflamm* 2015, 831059.
55. Schlegel, N., and Waschke, J. (2014). cAMP with other signaling cues converges on Rac1 to stabilize the endothelial barrier- a signaling pathway compromised in inflammation. *Cell Tissue Res* 355, 587-596.
56. Roberts, O.L., and Dart, C. (2014). cAMP signalling in the vasculature: the role of Epac (exchange protein directly activated by cAMP). *Biochem Soc Trans* 42, 89-97.
57. Wittchen, E.S., Aghajanian, A., and Burridge, K. (2011). Isoform-specific differences between Rap1A and Rap1B GTPases in the formation of endothelial cell junctions. *Small GTPases* 2, 65-76.
58. Singleton, P.A., Chatchavalvanich, S., Fu, P., Xing, J., Birukova, A.A., Fortune, J.A., Klibanov, A.M., Garcia, J.G., and Birukov, K.G. (2009). Akt-mediated transactivation of the S1P1 receptor in caveolin-enriched microdomains regulates endothelial barrier enhancement by oxidized phospholipids. *Circ Res* 104, 978-986.
59. Curry, F.R., and Adamson, R.H. (2013). Tonic regulation of vascular permeability. *Acta Physiol (Oxf)* 207, 628-649.
60. Givens, C., and Tzima, E. (2016). Endothelial Mechanosignaling: Does One Sensor Fit All? *Antioxid Redox Signal* 25, 373-388.
61. Lakshmikanthan, S., Zheng, X., Nishijima, Y., Sobczak, M., Szabo, A., Vasquez-Vivar, J., Zhang, D.X., and Chrzanowska-Wodnicka, M. (2015). Rap1 promotes endothelial mechanosensing complex formation, NO release and normal endothelial function. *EMBO Rep* 16, 628-637.
62. Liu, Y., Collins, C., Kiosses, W.B., Murray, A.M., Joshi, M., Shepherd, T.R., Fuentes, E.J., and Tzima, E. (2013). A novel pathway spatiotemporally activates Rac1 and redox signaling in response to fluid shear stress. *J Cell Biol* 201, 863-873.

63. Kurogane, Y., Miyata, M., Kubo, Y., Nagamatsu, Y., Kundu, R.K., Uemura, A., Ishida, T., Quertermous, T., Hirata, K., and Rikitake, Y. (2012). FGD5 mediates proangiogenic action of vascular endothelial growth factor in human vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 32, 988-996.
64. Ando, K., Fukuhara, S., Moriya, T., Obara, Y., Nakahata, N., and Mochizuki, N. (2013). Rap1 potentiates endothelial cell junctions by spatially controlling myosin II activity and actin organization. *J Cell Biol* 202, 901-916.
65. Wilson, C.W., Parker, L.H., Hall, C.J., Smyczek, T., Mak, J., Crow, A., Posthuma, G., De Maziere, A., Sagolla, M., Chalouni, C., et al. (2013). Rasip1 regulates vertebrate vascular endothelial junction stability through Epac1-Rap1 signaling. *Blood* 122, 3678-3690.
66. Post, A., Pannekoek, W.J., Ponsioen, B., Vliem, M.J., and Bos, J.L. (2015). Rap1 Spatially Controls ArhGAP29 To Inhibit Rho Signaling during Endothelial Barrier Regulation. *Mol Cell Biol* 35, 2495-2502.
67. de Kreuk, B.J., Gingras, A.R., Knight, J.D., Liu, J.J., Gingras, A.C., and Ginsberg, M.H. (2016). Heart of glass anchors Rasip1 at endothelial cell-cell junctions to support vascular integrity. *Elife* 5, e11394.
68. Liu, J.J., Stockton, R.A., Gingras, A.R., Ablooglu, A.J., Han, J., Bobkov, A.A., and Ginsberg, M.H. (2011). A mechanism of Rap1-induced stabilization of endothelial cell-cell junctions. *Mol Biol Cell* 22, 2509-2519.
69. Stockton, R.A., Shenkar, R., Awad, I.A., and Ginsberg, M.H. (2010). Cerebral cavernous malformations proteins inhibit Rho kinase to stabilize vascular integrity. *J Exp Med* 207, 881-896.
70. Pannekoek, W.J., Linnemann, J.R., Brouwer, P.M., Bos, J.L., and Rehmann, H. (2013). Rap1 and Rap2 antagonistically control endothelial barrier resistance. *PLoS One* 8, e57903.
71. Breslin, J.W., Zhang, X.E., Worthylake, R.A., and Souza-Smith, F.M. (2015). Involvement of local lamellipodia in endothelial barrier function. *PLoS One* 10, e0117970.
72. Daneshjou, N., Sieracki, N., van Nieuw Amerongen, G.P., Conway, D.E., Schwartz, M.A., Komarova, Y.A., and Malik, A.B. (2015). Rac1 functions as a reversible tension modulator to stabilize VE-cadherin trans-interaction. *J Cell Biol* 208, 23-32.
73. Walsh, T.G., Murphy, R.P., Fitzpatrick, P., Rochfort, K.D., Guinan, A.F., Murphy, A., and Cummins, P.M. (2011). Stabilization of brain microvascular endothelial barrier function by

shear stress involves VE-cadherin signaling leading to modulation of pTyr-occludin levels. *J Cell Physiol* 226, 3053-3063.

74. Siddiqui, M.R., Mayanil, C.S., Kim, K.S., and Tomita, T. (2015). Angiopoietin-1 Regulates Brain Endothelial Permeability through PTPN-2 Mediated Tyrosine Dephosphorylation of Occludin. *PLoS One* 10, e0130857.

75. Brown, M.D., and Sacks, D.B. (2006). IQGAP1 in cellular signaling: bridging the GAP. *Trends Cell Biol* 16, 242-249.

76. Tian, Y., Tian, X., Gawlak, G., Sarich, N., Sacks, D.B., Birukova, A.A., and Birukov, K.G. (2016). Role of IQGAP1 in endothelial barrier enhancement caused by OxPAPC. *Am J Physiol Lung Cell Mol Physiol*, ajplung.00095.02016.

77. Naikawadi, R.P., Cheng, N., Vogel, S.M., Qian, F., Wu, D., Malik, A.B., and Ye, R.D. (2012). A critical role for phosphatidylinositol (3,4,5)-trisphosphate-dependent Rac exchanger 1 in endothelial junction disruption and vascular hyperpermeability. *Circ Res* 111, 1517-1527.

78. Baumer, Y., Spindler, V., Werthmann, R.C., Bunemann, M., and Waschke, J. (2009). Role of Rac 1 and cAMP in endothelial barrier stabilization and thrombin-induced barrier breakdown. *J Cell Physiol* 220, 716-726.

79. Timmerman, I., Heemskerk, N., Kroon, J., Schaefer, A., van Rijssel, J., Hoogenboezem, M., van Unen, J., Goedhart, J., Gadella, T.W., Jr., Yin, T., et al. (2015). A local VE-cadherin and Trio-based signaling complex stabilizes endothelial junctions through Rac1. *J Cell Sci* 128, 3041-3054.

80. Boissier, P., and Huynh-Do, U. (2014). The guanine nucleotide exchange factor Tiam1: a Janus-faced molecule in cellular signaling. *Cell Signal* 26, 483-491.

81. Zhang, X.E., Adderley, S.P., and Breslin, J.W. (2016). Activation of RhoA, but Not Rac1, Mediates Early Stages of S1P-Induced Endothelial Barrier Enhancement. *PLoS One* 11, e0155490.

82. Phillips, M.J., Calero, G., Chan, B., Ramachandran, S., and Cerione, R.A. (2008). Effector proteins exert an important influence on the signaling-active state of the small GTPase Cdc42. *J Biol Chem* 283, 14153-14164.

83. Birukov, K.G., Bochkov, V.N., Birukova, A.A., Kawkitinarong, K., Rios, A., Leitner, A., Verin, A.D., Bokoch, G.M., Leitinger, N., and Garcia, J.G. (2004). Epoxycyclopentenone-

containing oxidized phospholipids restore endothelial barrier function via Cdc42 and Rac. *Circ Res* 95, 892-901.

84. Broman, M.T., Kouklis, P., Gao, X., Ramchandran, R., Neamu, R.F., Minshall, R.D., and Malik, A.B. (2006). Cdc42 regulates adherens junction stability and endothelial permeability by inducing  $\alpha$ -catenin interaction with the vascular endothelial cadherin complex. *Circ Res* 98, 73-80.
85. Kouklis, P., Konstantoulaki, M., Vogel, S., Broman, M., and Malik, A.B. (2004). Cdc42 regulates the restoration of endothelial barrier function. *Circ Res* 94, 159-166.
86. Kouklis, P., Konstantoulaki, M., and Malik, A.B. (2003). VE-cadherin-induced Cdc42 signaling regulates formation of membrane protrusions in endothelial cells. *J Biol Chem* 278, 16230-16236.
87. Barry, D.M., Xu, K., Meadows, S.M., Zheng, Y., Norden, P.R., Davis, G.E., and Cleaver, O. (2015). Cdc42 is required for cytoskeletal support of endothelial cell adhesion during blood vessel formation in mice. *Development* 142, 3058-3070.
88. Wang, B., Wylie, F.G., Teasdale, R.D., and Stow, J.L. (2005). Polarized trafficking of E-cadherin is regulated by Rac1 and Cdc42 in Madin-Darby canine kidney cells. *Am J Physiol Cell Physiol* 288, C1411-1419.
89. Qi, Y., Liu, J., Wu, X., Brakebusch, C., Leitges, M., Han, Y., Corbett, S.A., Lowry, S.F., Graham, A.M., and Li, S. (2011). Cdc42 controls vascular network assembly through protein kinase C $\alpha$  during embryonic vasculogenesis. *Arterioscler Thromb Vasc Biol* 31, 1861-1870.
90. Scheiermann, C., Frenette, P.S., and Hidalgo, A. (2015). Regulation of leucocyte homeostasis in the circulation. *Cardiovasc Res* 107, 340-351.
91. Nourshargh, S., and Alon, R. (2014). Leukocyte migration into inflamed tissues. *Immunity* 41, 694-707.
92. Ley, K., Laudanna, C., Cybulsky, M.I., and Nourshargh, S. (2007). Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol* 7, 678-689.
93. Nourshargh, S., Hordijk, P.L., and Sixt, M. (2010). Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. *Nat Rev Mol Cell Biol* 11, 366-378.
94. Heemskerk, N., van Rijssel, J., and van Buul, J.D. (2014). Rho-GTPase signaling in leukocyte extravasation: an endothelial point of view. *Cell Adh Migr* 8, 67-75.

95. Muller, W.A. (2016). Localized signals that regulate transendothelial migration. *Curr Opin Immunol* 38, 24-29.
96. Carman, C.V. (2009). Mechanisms for transcellular diapedesis: probing and pathfinding by 'invadosome-like protrusions'. *J Cell Sci* 122, 3025-3035.
97. Heemskerk, N., Schimmel, L., Oort, C., van Rijssel, J., Yin, T., Ma, B., van Unen, J., Pitter, B., Huveneers, S., Goedhart, J., et al. (2016). F-actin-rich contractile endothelial pores prevent vascular leakage during leukocyte diapedesis through local RhoA signalling. *Nat Commun* 7, 10493.
98. Woodfin, A., Voisin, M.B., Beyrau, M., Colom, B., Caille, D., Diapouli, F.M., Nash, G.B., Chavakis, T., Albelda, S.M., Rainger, G.E., et al. (2011). The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo. *Nat Immunol* 12, 761-769.
99. Martinelli, R., Zeiger, A.S., Whitfield, M., Sciuto, T.E., Dvorak, A., Van Vliet, K.J., Greenwood, J., and Carman, C.V. (2014). Probing the biomechanical contribution of the endothelium to lymphocyte migration: diapedesis by the path of least resistance. *J Cell Sci* 127, 3720-3734.
100. Luscinskas, F.W., Ma, S., Nusrat, A., Parkos, C.A., and Shaw, S.K. (2002). Leukocyte transendothelial migration: a junctional affair. *Semin Immunol* 14, 105-113.
101. Schaefer, A., and Hordijk, P.L. (2015). Cell-stiffness-induced mechanosignaling - a key driver of leukocyte transendothelial migration. *J Cell Sci* 128, 2221-2230.
102. Muller, W.A. (2016). Transendothelial migration: unifying principles from the endothelial perspective. *Immunol Rev* 273, 61-75.
103. Lessey-Morillon, E.C., Osborne, L.D., Monaghan-Benson, E., Guilluy, C., O'Brien, E.T., Superfine, R., and Burridge, K. (2014). The RhoA guanine nucleotide exchange factor, LARG, mediates ICAM-1-dependent mechanotransduction in endothelial cells to stimulate transendothelial migration. *J Immunol* 192, 3390-3398.
104. Sage, P.T., and Carman, C.V. (2009). Settings and mechanisms for trans-cellular diapedesis. *Front Biosci (Landmark Ed)* 14, 5066-5083.
105. Kanters, E., van Rijssel, J., Hensbergen, P.J., Hondius, D., Mul, F.P., Deelder, A.M., Sonnenberg, A., van Buul, J.D., and Hordijk, P.L. (2008). Filamin B mediates ICAM-1-driven leukocyte transendothelial migration. *J Biol Chem* 283, 31830-31839.



106. Schnoor, M., Lai, F.P., Zarbock, A., Klaver, R., Polaschegg, C., Schulte, D., Weich, H.A., Oelkers, J.M., Rottner, K., and Vestweber, D. (2011). Cortactin deficiency is associated with reduced neutrophil recruitment but increased vascular permeability in vivo. *J Exp Med* 208, 1721-1735.
107. Van Rijssel, J., Timmerman, I., Van Alphen, F.P., Hoogenboezem, M., Korchynskyi, O., Geerts, D., Geissler, J., Reedquist, K.A., Niessen, H.W., and Van Buul, J.D. (2013). The Rho-GEF Trio regulates a novel pro-inflammatory pathway through the transcription factor Ets2. *Biol Open* 2, 569-579.
108. van Buul, J.D., Allingham, M.J., Samson, T., Meller, J., Boulter, E., Garcia-Mata, R., and Burridge, K. (2007). RhoG regulates endothelial apical cup assembly downstream from ICAM1 engagement and is involved in leukocyte trans-endothelial migration. *J Cell Biol* 178, 1279-1293.
109. van Rijssel, J., Kroon, J., Hoogenboezem, M., van Alphen, F.P., de Jong, R.J., Kostadinova, E., Geerts, D., Hordijk, P.L., and van Buul, J.D. (2012). The Rho-guanine nucleotide exchange factor Trio controls leukocyte transendothelial migration by promoting docking structure formation. *Mol Biol Cell* 23, 2831-2844.
110. Alcaide, P., Newton, G., Auerbach, S., Sehrawat, S., Mayadas, T.N., Golan, D.E., Yacono, P., Vincent, P., Kowalczyk, A., and Luscinskas, F.W. (2008). p120-Catenin regulates leukocyte transmigration through an effect on VE-cadherin phosphorylation. *Blood* 112, 2770-2779.
111. Shaw, S.K., Bamba, P.S., Perkins, B.N., and Luscinskas, F.W. (2001). Real-time imaging of vascular endothelial-cadherin during leukocyte transmigration across endothelium. *J Immunol* 167, 2323-2330.
112. Schulte, D., Kuppers, V., Dartsch, N., Broermann, A., Li, H., Zarbock, A., Kamenyeva, O., Kiefer, F., Khandoga, A., Massberg, S., et al. (2011). Stabilizing the VE-cadherin-catenin complex blocks leukocyte extravasation and vascular permeability. *Embo j* 30, 4157-4170.
113. Vockel, M., and Vestweber, D. (2013). How T cells trigger the dissociation of the endothelial receptor phosphatase VE-PTP from VE-cadherin. *Blood* 122, 2512-2522.
114. Cook-Mills, J.M., Johnson, J.D., Deem, T.L., Ochi, A., Wang, L., and Zheng, Y. (2004). Calcium mobilization and Rac1 activation are required for VCAM-1 (vascular cell adhesion molecule-1) stimulation of NADPH oxidase activity. *Biochem J* 378, 539-547.



115. Mishra, R., and Singh, S.K. (2014). HIV-1 Tat C phosphorylates VE-cadherin complex and increases human brain microvascular endothelial cell permeability. *BMC Neurosci* 15, 80.
116. van Buul, J.D., Anthony, E.C., Fernandez-Borja, M., Burrridge, K., and Hordijk, P.L. (2005). Proline-rich tyrosine kinase 2 (Pyk2) mediates vascular endothelial-cadherin-based cell-cell adhesion by regulating beta-catenin tyrosine phosphorylation. *J Biol Chem* 280, 21129-21136.
117. Allingham, M.J., van Buul, J.D., and Burrridge, K. (2007). ICAM-1-mediated, Src- and Pyk2-dependent vascular endothelial cadherin tyrosine phosphorylation is required for leukocyte transendothelial migration. *J Immunol* 179, 4053-4064.
118. Cain, R.J., Vanhaesebroeck, B., and Ridley, A.J. (2010). The PI3K p110alpha isoform regulates endothelial adherens junctions via Pyk2 and Rac1. *J Cell Biol* 188, 863-876.
119. van Wetering, S., van Buul, J.D., Quik, S., Mul, F.P., Anthony, E.C., ten Klooster, J.P., Collard, J.G., and Hordijk, P.L. (2002). Reactive oxygen species mediate Rac-induced loss of cell-cell adhesion in primary human endothelial cells. *J Cell Sci* 115, 1837-1846.
120. van Wetering, S., van den Berk, N., van Buul, J.D., Mul, F.P., Lommerse, I., Mous, R., ten Klooster, J.P., Zwaginga, J.J., and Hordijk, P.L. (2003). VCAM-1-mediated Rac signaling controls endothelial cell-cell contacts and leukocyte transmigration. *Am J Physiol Cell Physiol* 285, C343-352.
121. Nottebaum, A.F., Cagna, G., Winderlich, M., Gamp, A.C., Linnepe, R., Polaschegg, C., Filippova, K., Lyck, R., Engelhardt, B., Kamenyeva, O., et al. (2008). VE-PTP maintains the endothelial barrier via plakoglobin and becomes dissociated from VE-cadherin by leukocytes and by VEGF. *J Exp Med* 205, 2929-2945.
122. Barzilai, S., Yadav, S.K., Morrell, S., Roncato, F., Klein, E., Stoler-Barak, L., Golani, O., Feigelson, S.W., Zemel, A., Nourshargh, S., et al. (2017). Leukocytes Breach Endothelial Barriers by Insertion of Nuclear Lobes and Disassembly of Endothelial Actin Filaments. *Cell Rep* 18, 685-699.
123. Mamdouh, Z., Kreitzer, G.E., and Muller, W.A. (2008). Leukocyte transmigration requires kinesin-mediated microtubule-dependent membrane trafficking from the lateral border recycling compartment. *J Exp Med* 205, 951-966.

124. Sullivan, D.P., Ruffer, C., and Muller, W.A. (2014). Isolation of the lateral border recycling compartment using a diaminobenzidine-induced density shift. *Traffic* 15, 1016-1029.
125. Weber, E.W., Han, F., Tauseef, M., Birnbaumer, L., Mehta, D., and Muller, W.A. (2015). TRPC6 is the endothelial calcium channel that regulates leukocyte transendothelial migration during the inflammatory response. *J Exp Med* 212, 1883-1899.
126. Mamdouh, Z., Mikhailov, A., and Muller, W.A. (2009). Transcellular migration of leukocytes is mediated by the endothelial lateral border recycling compartment. *J Exp Med* 206, 2795-2808.
127. Winger, R.C., Koblinski, J.E., Kanda, T., Ransohoff, R.M., and Muller, W.A. (2014). Rapid remodeling of tight junctions during paracellular diapedesis in a human model of the blood-brain barrier. *J Immunol* 193, 2427-2437.
128. Sidibe, A., and Imhof, B.A. (2014). VE-cadherin phosphorylation decides: vascular permeability or diapedesis. *Nat Immunol* 15, 215-217.
129. Reymond, N., d'Agua, B.B., and Ridley, A.J. (2013). Crossing the endothelial barrier during metastasis. *Nat Rev Cancer* 13, 858-870.
130. Locard-Paulet, M., Lim, L., Veluscek, G., McMahon, K., Sinclair, J., van Weverwijk, A., Worboys, J.D., Yuan, Y., Isacke, C.M., and Jorgensen, C. (2016). Phosphoproteomic analysis of interacting tumor and endothelial cells identifies regulatory mechanisms of transendothelial migration. *Sci Signal* 9, ra15.

## Figure legends

### Figure 1. The main transmembrane proteins in endothelial cell-cell junctions

Endothelial cells line the blood stream and are constantly exposed to fluid shear stress (top panel). The bottom panel shows the main transmembrane proteins in endothelial cell-cell junctions (right). They are associated with tight junctions or adherens junctions as indicated, with the exception of PECAM-1, which is not associated with either type of junction. Three different claudin, JAM and nectin genes are reported to be expressed in endothelial cells (numbers/letters indicated under protein name). Intracellular proteins that link transmembrane junctional proteins to the actin cytoskeleton (left). There are additional junctional molecules, such as CD99 and ESAM, which are omitted for clarity.

### Figure 2. Signaling molecules that regulate vascular permeability

The main junctional proteins that regulate vascular permeability are shown on the right cell. The left cell shows examples of receptors that increase vascular permeability, and the intracellular signaling molecules that contribute to this response, focusing on those regulated by Rho and Rap GTPases. ROS, reactive oxygen species.

### Figure 3. Signaling molecules that regulate leukocyte transendothelial migration

Leukocyte attachment to and transmigration across endothelial cells is a multistep process, involving a variety of different adhesion molecules on leukocytes and endothelial cells (top panel). During transendothelial migration, endothelial adhesion molecules ICAM-1, VCAM-1 and PECAM-1 interact with leukocytes and transmit signals into the endothelial cell (bottom panel), which promote the transmigration process. The left cell shows signals involving RhoG, Rac1 and Rap1 GTPases; the right cell shows signals involving RhoA. LBRC, lateral border recycling compartment; ROS, reactive oxygen species.

Figure 1

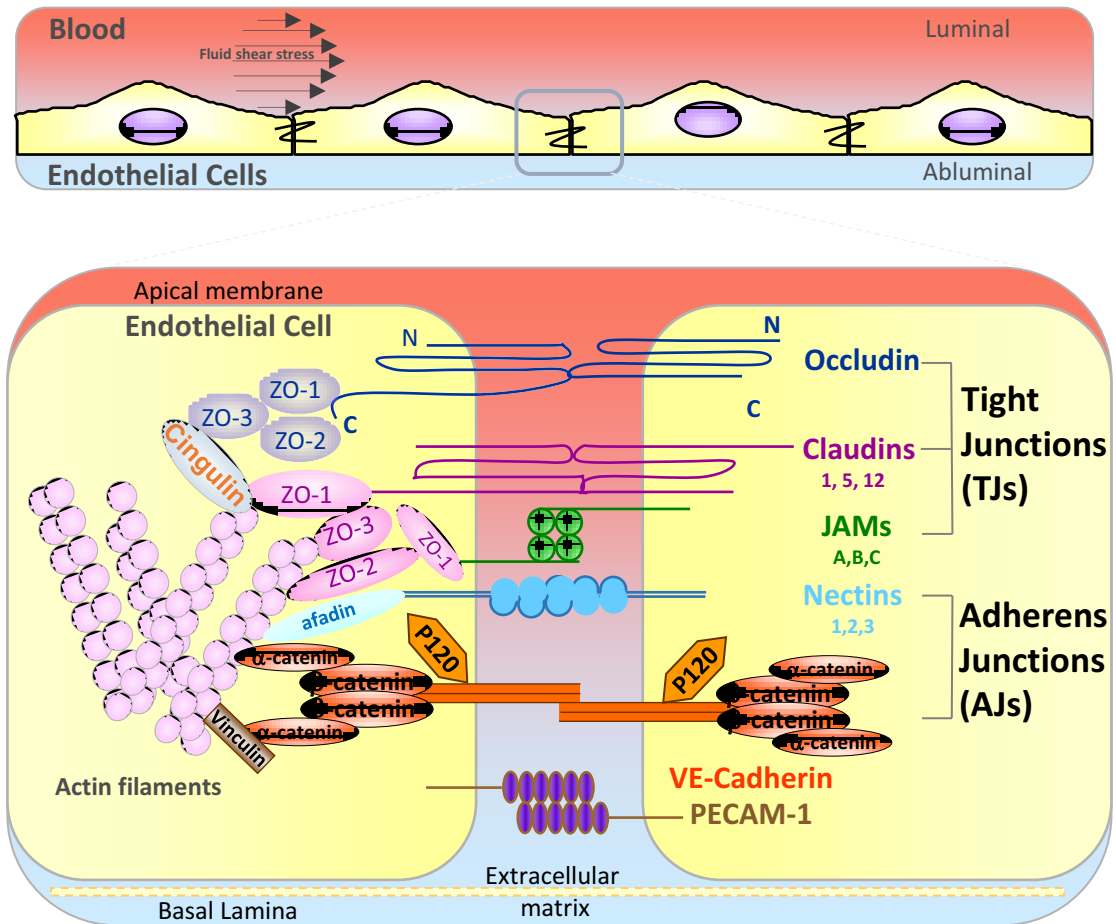


Figure 2

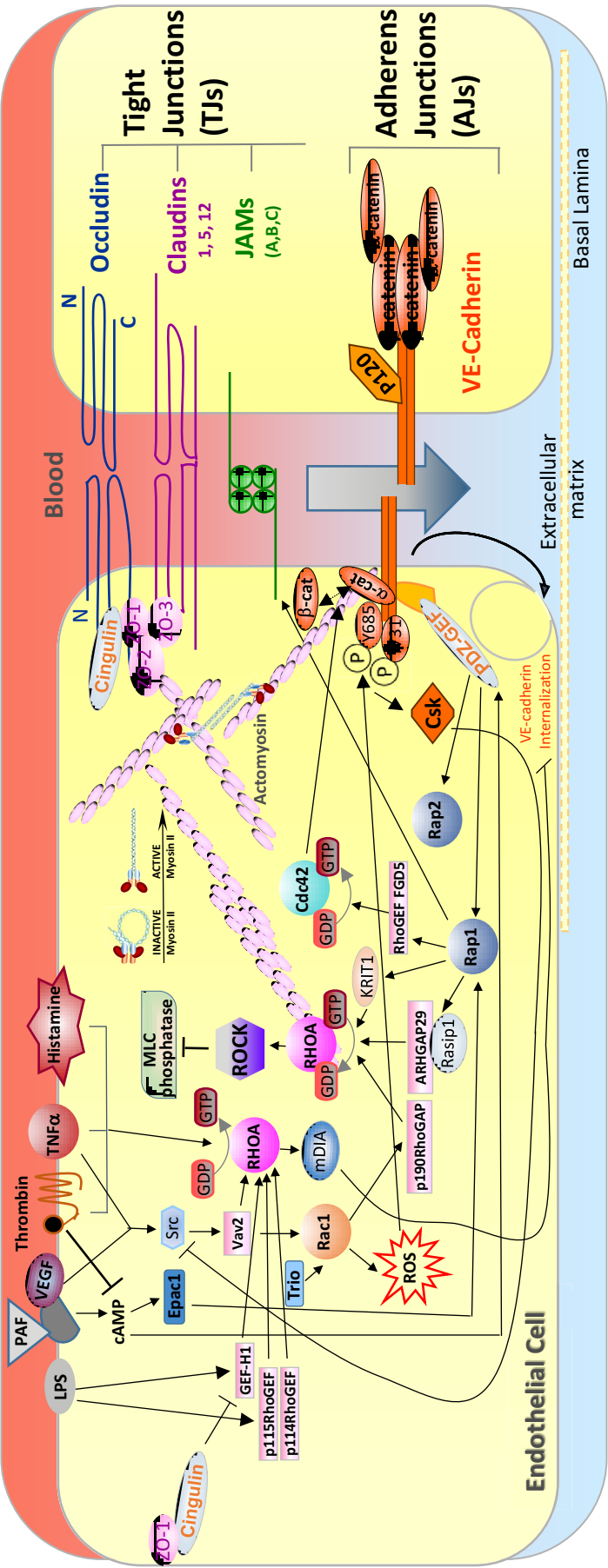


Figure 3

